MINIREVIEWS

Bacterial Resistance to Macrolide, Lincosamide, and Streptogramin Antibiotics by Target Modification

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Introduction. Macrolide, lincosamide, and streptogramin (MLS) antibiotics are chemically distinct but have a similar mode of action. They have a narrow spectrum of activity that includes gram-positive cocci (in particular, staphylococci, streptococci, and enterococci) and bacilli and gram-negative cocci. These drugs, especially clindamycin, are also potent against anaerobic bacteria. Gram-negative bacilli are usually resistant to MLS antibiotics, but certain enteric bacilli and *Haemophilus* spp. are susceptible to azithromycin in vitro. However, the activities of these antibiotics against *Campylobacter*, *Legionella*, and *Chlamydia* spp. are at the origin of the recent renaissance of erythromycin.

Macrolides are composed of a minimum of two amino and/or neutral sugars attached to a lactone ring of variable size. Macrolides commercially available or in clinical development can be divided into 14-, 15-, and 16-membered lactone ring macrolides. These classes differ in their pharmacokinetic properties and in their responses to bacterial resistance mechanisms. Lincosamides (lincomycin and the more active semisynthetic derivative clindamycin) are alkyl derivatives of proline and are devoid of a lactone ring. Streptogramin antibiotics are used in clinical practice in certain countries, including Belgium and France. They are composed of two factors, A and B (II and I in pristinamycin and M and S in virginiamycin, respectively), that act in synergy and are produced by the same microorganism.

In 1956, a few years after the introduction of erythromycin in therapy, resistance of staphylococci to this drug emerged and subsequently spread in France, the United Kingdom, and the United States (13, 22, 37). The MLS cross-resistance phenotype due to modification of the drug target is widely distributed and has, since then, been detected in *Staphylococcus* spp. (41, 72), *Streptococcus* spp. (18, 23, 30), *Enterococcus* spp. (16), *Corynebacterium diptheriae* (17), *Bacteroides* spp. (61, 65), *Clostridium* spp. (64, 79), *Bacillus* spp. (47, 48), *Lactobacillus* spp. (5, 57), *Mycoplasma pneumoniae* (69), *Campylobacter* spp. (11) and, recently, *Propionibacterium* spp. (20) and members of the family *Enterobacteriaceae* (1).

This paper reviews the biochemical mechanism and the genetic basis of resistance to MLS antibiotics in human pathogens by target modification.

Biochemical mechanism. Clinical isolates resistant to erythromycin synthesize an enzyme that N^6 dimethylates an adenine residue in 23S rRNA (40). The precise site of methylation has been located in a highly conserved sequence in *Staphylococcus aureus* strains harboring *ermA*, *ermB*, and *ermC* genes conferring MLS resistance (73) and in a strain of *Bacillus stearothermophilus* harboring the methylase of *Streptomyces erythreus*, an erythromycin producer (66). In a strain of *Escherichia coli* containing *ermC*, the modified adenine residue is at position 2058 in 23S rRNA (66). rRNA methylation probably leads to a conformational change in the ribosome that leads to coresistance to macrolides, lincosamides, and streptogramin B-type antibiotics, probably because the binding sites of these drugs overlap (21). Streptogramin A-type antibiotics are unaffected and synergy between the two components of streptogramin against MLS-resistant strains is maintained (14).

Genetic determinants of resistance. A sequence comparison of *erm* (erythromycin resistance methylase) genes from various bacterial species and results of hybridization experiments under stringent conditions led to the distinction of a minimum of eight classes of resistance determinants (2, 55) (Table 1). The prototype genes for these classes were isolated from the human pathogens *S. aureus* (*ermA* and *ermC*), *Streptococcus sanguis* (*ermAM*), and *Bacteroides fragilis* (*ermF*), from the soil bacteria *Bacillus licheniformis* (*ermD*) and *Bacillus sphaericus* (*ermG*), and from two erythromycin producers, *S. erythreus* (*ermE*) and *Arthrobacter* sp. (*ermA'*). The amino acid sequences of the methylases encoded by these determinants are related, indicating that the *erm* genes are derived from a common ancestor, possibly belonging to an antibiotic producer (2). However, various degrees of similarity among the enzymes can be observed (2). The sequences of the prototype methylases ErmA, ErmC, and ErmAM from the human pathogens are closely related (greater than 50% similarity) and form a group (2), whereas ErmF is more distant (55). The determinants of the other clinical isolates studied can be assigned to four hybridization classes: *ermA*, *ermC*, *ermAM*, and *ermF*. This gene distribution is relatively species specific (Table 2). *ermA* and *ermC* are common in staphylococci and are, respectively, often located in the chromosome and on plasmids; *ermC* has, in addition to being detected in staphylococci, also been detected in *Bacillus subtilis* (47), *Lactobacillus* sp. (57), and enterobacteria (44). *ermF* is present in various *Bacteroides* spp. The *ermAM* gene is widespread in streptococci, pneumococci, and enterococci, in which it is often borne by transposons, such as Tn917 (74) and Tn1545 (15). However, because of horizontal transfer of genetic information, this determinant is not confined to these microorganisms. Closely related sequences (designated *ermB* in transposon Tn551) have been found in *S. aureus* (78), in which they are apparently rare (36). Determinants related to *ermAM* have

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TABLE 1. Classes of *erm* genes

Gene	Bacterial species	Localization	Reference
ermA	Staphylococcus aureus	Tn554	49
ermAM	Streptococcus sanguis	pAM77	30
ermC	Staphylococcus aureus	pE194	31
ermD	Bacillus licheniformis	Chromosome	24
ermE	Streptomyces erythreus	Chromosome	75
ermA'	Arthrobacter sp.	Chromosome	58
ermF	Bacteroides fragilis	pBF4	55
ermG	Bacillus sphaericus	Chromosome	48

been detected recently in *Clostridium perfringens*, *Clostridium difficile*, and enterobacteria, in which they are designated *ermP*, *ermZ*, and *ermBC*, respectively (8, 10, 26), and also in *Lactobacillus reuteri* (5). The emergence of this gene class in enterobacteria has provided additional evidence for the existence of cross-transfer of genetic material from gram-positive to gram-negative bacteria under natural conditions (10). There are other *erm* determinants that have not yet been identified (72). Despite the high degree of nucleotide sequence diversity for rRNA methylases, the polymerase chain reaction, done with primers that are complementary to conserved regions in the *erm* genes, allows the detection of MLS resistance in both gram-positive and gram-negative bacteria (4).

Regulation of MLS resistance in gram-positive cocci. Expression of MLS resistance in staphylococci may be constitutive or inducible. When expression is constitutive, the strains are resistant to all macrolides, lincosamides, and

TABLE 2. Distribution of *erm* genes in clinically important bacterial species

| Hybridization class	Gene	Host	Reference(s)
ermA	ermA	Staphylococcus aureus	49, 72
		Coagulase-negative staphylococci	72
ermAM	ermP	Clostridium perfringens	8
	ermZ	C. difficile	26
		Enterococcus faecalis	23, 42, 78
	ermBC	Escherichia coli	10
		Lactobacillus reuteri	5
	ermAM	Streptococcus sanguis	30
		S. pneumoniae	52, 78
		S. agalactiae and S. pyogenes	23, 52, 78
ermC	ermB	S. aureus	78
		Bacillus subtilis	47
		Lactobacillus spp.	57
	ermC	S. aureus	31, 72
		Coagulase-negative staphylococci	72
	ermM	S. epidermidis	41
ermF	ermF	Bacteroides fragilis	55
		B. ovatus	68
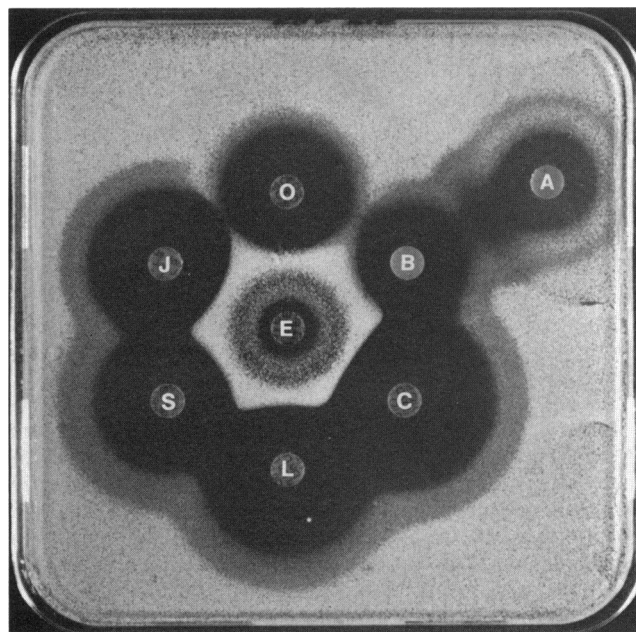
FIG. 1. Disk agar susceptibility test of an *S. aureus* isolate with inducible MLS resistance. C, clindamycin (2 U); E, erythromycin (15 U); J, josamycin (100 μg); L, lincomycin (15 μg); O, oleandomycin (15 U); S, spiramycin (100 μg); A, streptogramin A (20 μg); B, streptogramin B (40 μg).

streptogramin B-type antibiotics. Streptogramin A-type antibiotics escape resistance, and synergy with streptogramin B-type antibiotics is retained (14). The bactericidal activity of the streptogramin complex, however, can be altered (14). When expression is inducible, the strains are resistant to 14-membered (erythromycin, roxithromycin, and often oleandomycin) and 15-membered (azithromycin, which is under development) macrolides only. The 16-membered macrolides (spiramycin, josamycin, miocamycin, and midecamycin), the commercially available lincosamides, and the streptogramin antibiotics remain active. This dissociated resistance is due to differences in the inducing abilities of MLS antibiotics; only 14- and 15-membered macrolides are effective inducers of methylase synthesis. In agar disk diffusion tests, D-shaped inhibition zones around disks impregnated with a noninducing macrolide, a lincosamide, or a streptogramin B-type antibiotic can be observed if a disk of erythromycin is placed nearby (Fig. 1).

The inducible or constitutive character of resistance is not related to the class of *erm* determinant but depends on the sequence of the regulatory region upstream from the structural gene for the methylase. The regulation of expression of the *ermC* determinant from staphylococcal plasmid pE194 has been extensively studied and is explained by a translation attenuation mechanism (31; for reviews see references 7, 19, and 77). Adjacent to the *ermC* structural gene for the methylase is an open reading frame encoding a 14-amino-acid control peptide. Both genes are cotranscribed in a single mRNA (Fig. 2). Translation of this mRNA implies that the ribosomes recognize initiation sequences for the two open reading frames. These sequences, designated ribosome binding sites or Shine-Dalgarno (SD) sequences, are separated by a few base pairs from the initiation codons. In the case of *ermC* (Fig. 2, conformation I), the 5' end of the correspond-
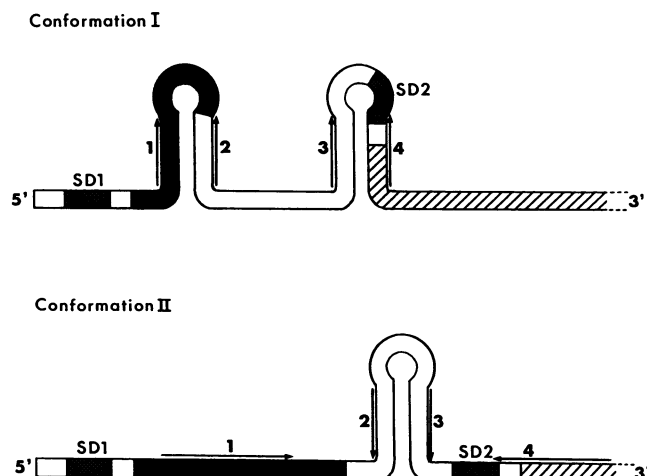
Conformation I

Conformation II

FIG. 2. Alternative conformations of the mRNA from the inducible *ermC* gene from pNE194. Shown are the secondary structures of the mRNA (☐) in the absence (conformation I) or in the presence (conformation II) of erythromycin. Symbols: ■, sequence of the control peptide; ▨, sequence of the methylase. 1, 2, 3, and 4, represent inverted repeats (77).

ing mRNA presents a set of four inverted repeats which sequester, by base pairing in the absence of erythromycin, SD2 and the initiation codon for the methylase. The latter are therefore not accessible to the ribosomes, and only the sequence corresponding to the control peptide is translated through region 1, which is not an impediment. When present, erythromycin binds to ribosomes, including those involved in the synthesis of the control peptide, and causes them to stall. Ribosome stalling probably induces conformational rearrangements in the mRNA and causes displacement of the stem-loop structure (Fig. 2, conformation II). Being free, SD2 can then be recognized by ribosomes for the initiation of translation of the methylase. The methylase is synthesized by ribosomes that are efficient for protein synthesis and that are not complexed to erythromycin or that are methylated because of the low-level production of enzyme allowed, in the absence of erythromycin, by spontaneous and transitory mRNA rearrangements (19). The activation of mRNA is followed by an increase in its half-life that enhances enzyme synthesis; this stabilization appears to be due to a stalling of ribosomes that protects the transcripts from degradation by RNases (62). In this model, regulation is due, in part, to a methylation-mediated feedback: when all of the ribosomes are methylated, stalling does not occur and mRNA molecules return to the inactive conformation. Regulation at the level of translation has also been demonstrated: SD2 in mRNA and the binding site for the methylase in 23S rRNA have structural similarities; the *ermC*-encoded methylase, when in excess, may thus bind to SD2, blocking partly its own production (9). A similar model has also been proposed for the regulation of the inducible determinants *ermA* of staphylococcal transposon Tn*554* (49) and *ermG* of *B. sphaericus* (48). However, the regulatory regions of these determinants are more complex, containing two short control peptides, and induction involves a series of rearrangements of the inverted repeats. In addition, erythromycin may cause the induction of the inducible chloramphenicol resistance *cat-86* gene, which is also controlled by a translational attenuator structure. Evidence has recently been

presented that the requisite ribosome stalling in the leader region required for the expression of both *erm* and *cat* genes is determined by complementarity between the 16S rRNA sequence and the sequence at the stall site in the mRNA of the two genes (59).

In staphylococci, generally only 14- and 15-membered macrolides are effective as inducers. The specificity of induction is thought to be related to the mode of action of the various macrolides; noninducing macrolides can provoke a premature release (or inappropriate stalling) of the ribosomes that will not allow mRNA to refold into an active structure (77). The amino acid composition of the control peptide (45) and host factors (29), probably ribosomal structures, are also responsible for the specificity of induction. Changes in induction specificity have been reported in mutants of *S. aureus* obtained in vitro by antibiotic selection; lincosamides can act as inducers in these strains (71). Constitutive expression can be obtained from inducible strains at frequencies of 10^{-7} to 10^{-8} by selection on agar plates containing inhibitory concentrations of noninducing macrolide, lincosamide, or streptogramin B-type antibiotics. The variants obtained exhibit point mutations that decrease the stability of the stem-loop structure sequestering SD2, deletions in the leader peptide region, or direct tandem duplications of repeated segments (27). In clinical isolates, constitutive MLS resistance is also explained by the structure of the regulatory region; constitutive expression of *ermC* in *B. subtilis* (47) and in *Staphylococcus epidermidis* (41) is due to deletions which leave SD1 separated from SD2 by a few base pairs only. Selection of a constitutive mutant during clindamycin therapy of an infection caused by an inducibly resistant strain of *S. aureus* has been reported (76).

MLS resistance in streptococci can also be expressed constitutively or inducibly. However, unlike the case in staphylococci, various macrolides or lincosamides may act as inducers. This fact explains the diversity of resistance phenotypes observed by agar disk diffusion (32) and the zonal resistance to lincomycin observed mainly in beta-hemolytic streptococci (18). Thus, in streptococci, whether inducible or constitutive, resistance by ribosomal methylation is crossed among macrolides, lincosamides, and streptogramin B-type antibiotics. In *S. sanguis*, the peculiar features of induction are explained by the complex conformation of the regulatory region upstream from the *ermAM* gene (30).

Genetic basis of MLS resistance in gram-positive cocci. In staphylococci, inducible and constitutive MLS resistance is often borne by small, nonconjugative multicopy plasmids of 2 to 4 kb containing the *ermC* determinant and conferring resistance to MLS antibiotics only. Plasmid pE194 in this family has been extensively studied (77). In both *S. aureus* and coagulase-negative staphylococci, MLS resistance appears exclusively mediated by plasmids related to pNE131 (72), a 2.3-kb plasmid first detected in *S. epidermidis* (41). Larger, conjugative plasmids are rarely found; a 28.2-kb plasmid detected in Japan in 1960 and encoding a penicillinase also carries transposon Tn*551*, which includes the constitutive *ermB* determinant (46, 51). This transposable element is structurally related to the enterococcal transposon Tn*917* (74), and both belong to the Tn*3* family (38). Although borne by a self-transferable plasmid, Tn*551* is rarely found in staphylococci. Another transposon, Tn*554*, detected in the chromosome of *S. aureus* (53), confers inducible MLS resistance. This 6.7-kb element, which also encodes resistance to spectinomycin, exhibits peculiar properties; it does not possess inverted or directly repeated sequences at its

termini, it does not generate a duplication of target DNA upon insertion, and it is inserted in the same orientation preferentially at a single site in the chromosome (50). Tn554-like sequences have spread in clinical isolates of staphylococci and account for chromosomal resistance to MLS antibiotics (72). Transfer of resistance among staphylococci can occur by conjugation and transformation in addition to transduction, which was believed, for many years, to be the only mechanism for the exchange of genetic information among these organisms. As already mentioned, the small MLS resistance plasmids are not conjugative, and their transfer relies on mobilization by coresident conjugative plasmids. Conjugation requires forced cell-to-cell contact obtained by mating on filters. Under natural conditions, plasmid transfer is believed to occur by a process referred to as phage-mediated conjugation (39). Prophage genes in either the donor or the recipient facilitate plasmid transfer.

Since the first report of plasmid-mediated resistance to MLS antibiotics in *Enterococcus faecalis* (16), MLS resistance plasmids have been found in nearly all the *Streptococcus* spp. examined, with the notable exception of *Streptococcus pneumoniae* (for a review, see reference 28). In *E. faecalis* and in streptococci of groups A, B, C, and G, plasmids conferring MLS resistance alone or associated with chloramphenicol resistance range in size from 25 to 30 kb and are present at low copy numbers per cell. These structurally related conjugative plasmids have broad host ranges. As in staphylococci, the transfer of MLS resistance occurs by forced cell-to-cell contact on solid medium rather than in broth, although conjugative transfer was first reported in liquid medium (35). Larger plasmids, ranging in size from 115 to 150 kb and bearing additional resistance markers (i.e., chloramphenicol, kanamycin, streptomycin, and tetracycline), have been detected in *E. faecalis* and *Enterococcus faecium* (28). pAM77, from *S. sanguis*, is a small, 6.7-kb plasmid unusual in this bacterial genus (80). Transposable erythromycin resistance has been demonstrated in *E. faecalis*. Tn917 is a 5.3-kb element identified on the 22-kb multiple resistance plasmid pAD2 (74). As already mentioned, this transposon belongs to the Tn3 family and mediates inducible resistance to macrolides; interestingly, erythromycin induces transposition of the element together with MLS resistance (74). Tn3871, from plasmid pJH1 of *E. faecalis*, is indistinguishable from Tn917 (6). Sequences that hybridize with Tn3871 are present in plasmids of enterococci isolated from animal and human origins in various geographical areas in the United States, indicating a wide distribution of Tn917-like elements (60). The plasmids vary in size from 26 to 105 kb and also contain streptomycin and kanamycin resistance determinants.

Transfer of MLS resistance in the absence of plasmid DNA has been demonstrated in *S. pneumoniae*, *Streptococcus bovis*, and oral and group A, B, F, and G streptococci (12, 33). None of the multiresistant strains of *S. pneumoniae* studied so far harbors a resistance plasmid. In *S. pneumoniae* BM4200, the *ermAM* gene, together with the tetracycline and kanamycin resistance genes *tetM* and *aphA3*, respectively, is part of the conjugative transposon Tn1545 (15). This 25.3-kb element is self-transferable by conjugation to a wide variety of gram-positive bacteria, in which it is able to be transposed into various sites (15). Unlike most transposons, Tn1545 is not flanked by terminal inverted repeated sequences, does not possess variable base pairs at its extremities, and does not generate a duplication of the target DNA upon insertion. A 67-kb element conferring resistance to MLS, chloramphenicol, and tetracycline has been identi-

fied in the chromosome of *Streptococcus agalactiae* B109 and also shown to be transferred by a conjugationlike mechanism (34).

Transduction in beta-hemolytic streptococci and transformation in pneumococci may also play a role in the dissemination of antibiotic resistance (28).

Genetic basis of MLS resistance in other species. Plasmid-mediated resistance to MLS antibiotics in the gram-negative anaerobic *Bacteroides* spp. was first documented in 1975 (61). The phenotype is usually constitutive, but inducibility has recently been reported (56). Self-transferable plasmids that differ in size have been isolated: pBFTM10 and pCP1 (14.6 kb), which are probably identical (25, 70); pIP410 (41 kb) from *B. fragilis* (54), renamed pBF4; and pBI136 (82 kb) from *Bacteroides ovatus* (67, 68). The MLS resistance determinants of these plasmids are closely related, and those of pBFTM10 and pIP410 are part of transposons Tn4400 and Tn4351, respectively (65). Certain resistance genes are apparently located in the chromosomes of plasmid-free strains and can be transferred by a conjugationlike mechanism (65).

In *Clostridium* spp., constitutive and inducible MLS resistance, either plasmid (8) or chromosome (26) mediated, is found in animal and human strains.

Certain *C. diphtheriae* strains and diphtheroids also harbor plasmids conferring resistance to erythromycin (63).

In a strain of *E. coli* recently identified as a "new" host for MLS resistance, the *ermBC* determinant is carried, together with the *ereB* gene, responsible for erythromycin inactivation, by a 150-kb self-transferable plasmid (3, 10).

Conclusion. Study since 1956 (13) of inducible MLS resistance turned out to be extremely rewarding. Elucidation of the biochemical mechanism of resistance led to the understanding of bacterial resistance to structurally unrelated drugs. Discovery of translational attenuation (a feature that appears to be more common in nature than positive or negative regulation at the level of transcription) as the basis for the inducibility of resistance explained the ease in obtaining constitutive mutants and explained why macrolides can induce gene transposition (74) and resistance to other families of antibiotics (59). Sequence analysis of the structural genes for rRNA methylases revealed a high degree of genetic diversity for a single biochemical mechanism (Table 2), a favorable situation for the study of molecular evolution (2). Finally, the latter approach also provided additional evidence for cross-transfer of DNA among prokaryotes in nature (10).

As reported in the accompanying review (43), the results obtained at the molecular level are of great help for the routine detection of bacterial resistance to MLS antibiotics.

REFERENCES

1. **Arthur, M., A. Andremont, and P. Courvalin.** 1987. Distribution of erythromycin esterase and RNA methylase genes in members of the family *Enterobacteriaceae* highly resistant to erythromycin. Antimicrob. Agents Chemother. **31:**404–409.
2. **Arthur, M., A. Brisson-Noël, and P. Courvalin.** 1987. Origin and evolution of genes specifying resistance to macrolide, lincosamide and streptogramin antibiotics: data and hypotheses. J. Antimicrob. Chemother. **20:**783–802.
3. **Arthur, M., and P. Courvalin.** 1986. Contribution of two different mechanisms to erythromycin resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **30:**694–700.
4. **Arthur, M., C. Molinas, C. Mabilat, and P. Courvalin.** 1990.

Detection of erythromycin resistance by the polymerase chain reaction using primers in conserved regions of *erm* rRNA methylase genes. Antimicrob. Agents Chemother. **34**:2024–2026.

5. **Axelsson, L. T., S. I. Ahrné, M. C. Andersson, and S. R. Stahl.** 1988. Identification and cloning of a plasmid-encoded erythromycin resistance determinant from *Lactobacillus reuteri*. Plasmid **20**:171–174.

6. **Banai, M., and D. J. LeBlanc.** 1984. *Streptococcus faecalis* R plasmid pJH1 contains an erythromycin resistance transposon (Tn*3871*) similar to transposon Tn*917*. J. Bacteriol. **158**:1172–1174.

7. **Bechhofer, D. H.** 1990. Triple post-transcriptional control. Mol. Microbiol. **4**:1419–1423.

8. **Berryman, D. I., and J. I. Rood.** 1989. Cloning and hybridization analysis of *ermP*, a macrolide-lincosamide-streptogramin B resistance determinant from *Clostridium perfringens*. Antimicrob. Agents Chemother. **33**:1346–1353.

9. **Breidt, F., and D. Dubnau.** 1990. Identification of *cis*-acting sequences required for translational autoregulation of the *ermC* methylase. J. Bacteriol. **172**:3661–3668.

10. **Brisson-Noël, A., M. Arthur, and P. Courvalin.** 1988. Evidence for natural gene transfer from gram-positive cocci to *Escherichia coli*. J. Bacteriol. **170**:1739–1745.

11. **Burridge, R., C. Warren, and I. Phillips.** 1986. Macrolide, lincosamide and streptogramin resistance in *Campylobacter jejuni/coli*. J. Antimicrob. Chemother. **17**:315–321.

12. **Buu-Hoï, A., and T. Horodniceanu.** 1980. Conjugative transfer of multiple antibiotic resistance markers in *Streptococcus pneumoniae*. J. Bacteriol. **142**:313–320.

13. **Chabbert, Y. A.** 1956. Antagonisme *in vitro* entre l'érythromycine et la spiramycine. Ann. Inst. Pasteur (Paris) **90**:787–790.

14. **Chabbert, Y. A., and P. Courvalin.** 1971. Synergie des composants des antibiotiques du groupe de la streptogramine. Pathol. Biol. **19**:613–619.

15. **Courvalin, P., and C. Carlier.** 1986. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. Mol. Gen. Genet. **205**:291–297.

16. **Courvalin, P., C. Carlier, and Y. A. Chabbert.** 1972. Plasmid linked tetracycline and erythromycin resistance in group D *Streptococcus*. Ann. Inst. Pasteur (Paris) **123**:755–759.

17. **Coyle, M. B., B. H. Minshen, J. A. Bland, and P. C. Hsu.** 1979. Erythromycin and clindamycin resistance in *Corynebacterium diphteriae* from skin lesions. Antimicrob. Agents Chemother. **16**:525–527.

18. **Dixon, J. M. S., and A. E. Lipinski.** 1974. Infections with β-hemolytic *Streptococcus* resistant to lincomycin and erythromycin and observations on zonal-pattern resistance to lincomycin. J. Infect. Dis. **130**:351–356.

19. **Dubnau, D.** 1984. Translational attenuation: the regulation of bacterial resistance to the macrolide-lincosamide-streptogramin B antibiotics. Crit. Rev. Biochem. **16**:103–132.

20. **Eady, E. A., J. I. Ross, J. H. Cove, K. T. Holland, and W. J. Cunliffe.** 1989. Macrolide-lincosamide-streptogramin B (MLS) resistance in cutaneous propionibacteria: definition of phenotypes. J. Antimicrob. Chemother. **23**:493–502.

21. **Fernandez-Munoz, R., R. E. Monro, R. Torres-Pinedo, and D. Vasquez.** 1971. Substrate- and antibiotic-binding sites at the peptidyl-transferase centre of *Escherichia coli* ribosomes. Studies on the chloramphenicol, lincomycin and erythromycin sites. Eur. J. Biochem. **23**:185–193.

22. **Garrod, L. P.** 1967. The erythromycin group of antibiotics. Br. Med. J. **2**:57–63.

23. **Gilmore, M. S., D. Behnke, and J. J. Ferretti.** 1982. Evolutionary relatedness of MLS resistance and replication function sequences on streptococcal antibiotic resistance plasmids, p. 174–176. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.

24. **Gryczan, T. J., M. Israeli-Reches, M. Del Blue, and D. Dubnau.** 1984. DNA sequence and regulation of *ermD*, a macrolide-lincosamide-streptogramin B resistance element from *Bacillus licheniformis*. Mol. Gen. Genet. **194**:349–356.

25. **Guiney, D. G., P. Hasegawa, D. Stalker, and C. E. Davis.** 1984.

Homology between clindamycin resistance plasmids in *Bacteroides fragilis*. Plasmid **11**:268–271.

26. **Hächler, H., B. Berger-Bächi, and F. H. Kayser.** 1987. Genetic characterization of a *Clostridium difficile* erythromycin-clindamycin resistance determinant that is transferable to *Staphylococcus aureus*. Antimicrob. Agents Chemother. **31**:1039–1045.

27. **Hahn, S., G. Grandi, T. T. Gryczan, and D. Dubnau.** 1982. Translational attenuation of *ermC*: a deletion analysis. Mol. Gen. Genet. **186**:204–216.

28. **Horaud, T., C. Le Bouguénec, and K. Pepper.** 1985. Molecular genetics of resistance to macrolides, lincosamides and streptogramin B (MLS) in streptococci. J. Antimicrob. Chemother. **16**:111–135.

29. **Horinouchi, S., and T. Beppu.** 1985. Construction and application of a promoter-probe plasmid that allows chromogenic identification in *Streptomyces lividans*. J. Bacteriol. **162**:406–412.

30. **Horinouchi, S., W. H. Byeon, and B. Weisblum.** 1983. A complex attenuator regulates inducible resistance to macrolides, lincosamides, and streptogramin type B antibiotics in *Streptococcus sanguis*. J. Bacteriol. **154**:1252–1262.

31. **Horinouchi, S., and B. Weisblum.** 1980. Post-transcriptional modification of RNA conformation: mechanism that regulates erythromycin-induced resistance. Proc. Natl. Acad. Sci. USA **7**:7079–7083.

32. **Horodniceanu, T., L. Bougueleret, and F. Delbos.** 1979. Phenotypic aspects of resistance to macrolide and related antibiotics in β-haemolytic group A, B, C and G streptococci, P. 122–131. *In* R. Facklam, G. Laurell, and I. Lind (ed.), Recent developments in laboratory identification techniques. Excerpta Medica, Amsterdam.

33. **Horodniceanu, T., C. Le Bouguenec, A. Buu-Hoï, and G. Bieth.** 1982. Conjugative transfer of antibiotic resistance markers in beta-hemolytic streptococci in the presence and absence of plasmid DNA, p. 105–108. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.

34. **Inamine, J. M., and V. Burdett.** 1985. Structural organization of a 67-kilobase streptococcal conjugative element mediating multiple antibiotic resistance. J. Bacteriol. **161**:620–626.

35. **Jacob, A. E., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117**:360–372.

36. **Jenssen, W. D., S. Thakker-Varia, D. T. Dubin, and M. P. Weinstein.** 1987. Prevalence of macrolide-lincosamide-streptogramin B resistance and *erm* gene classes among clinical strains of staphylococci and streptococci. Antimicrob. Agents Chemother. **31**:883–888.

37. **Jones, W. F., R. L. Nichols, and M. Finland.** 1966. Development of resistance and cross-resistance in vitro to erythromycin, carbomycin, oleandomycin and streptogramin. Proc. Soc. Exp. Biol. Med. **93**:388–393.

38. **Khan, S., and R. P. Novick.** 1980. Terminal nucleotide sequences of Tn*551*, a transposon specifying erythromycin resistance in *Staphylococcus aureus*: homology with Tn*3*. Plasmid **4**:148–154.

39. **Lacey, R. W.** 1980. Evidence for two mechanisms of plasmid transfer in mixed cultures of *Staphylococcus aureus* in vitro. J. Gen. Microbiol. **119**:432–435.

40. **Lai, C. J., and B. Weisblum.** 1971. Altered methylation of ribosomal RNA in an erythromycin-resistant strain of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **68**:856–860.

41. **Lampson, B. C., and J. T. Parisi.** 1986. Naturally occurring *Staphylococcus epidermidis* plasmid expressing constitutive macrolide-lincosamide-streptogramin B resistance contains a deleted attenuator. J. Bacteriol. **166**:479–483.

42. **LeBlanc, D. J., J. M. Inamine, and L. N. Lee.** 1986. Broad geographical distribution of homologous erythromycin, kanamycin, and streptomycin resistance determinants among group D streptococci of human and animal origin. Antimicrob. Agents Chemother. **29**:549–555.

43. **Leclercq, R., and P. Courvalin.** 1991. Intrinsic and unusual

resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria. Antimicrob. Agents Chemother. **35**:1273–1276.

44. **Mabilat, C., and P. Courvalin.** 1988. Gene heterogeneity for resistance to macrolides, lincosamides and streptogramins in *Enterobacteriaceae.* Ann. Inst. Pasteur (Paris) **139**:677–681.

45. **Mayford, M., and B. Weisblum.** 1990. The *ermC* leader peptide: amino acid alterations leading to differential efficiency of induction by macrolide-lincosamide-streptogramin B antibiotics. J. Bacteriol. **172**:3772–3779.

46. **Mitsuhashi, S., M. Morimura, K. Kono, and H. Oshima.** 1963. Elimination of drug resistance of *Staphylococcus aureus* by treatment with acriflavine. J. Bacteriol. **86**:162–164.

47. **Monod, M., C. Denoya, and D. Dubnau.** 1986. Sequence and properties of pIM13, a macrolide-lincosamide-streptogramin B resistance plasmid from *Bacillus subtilis.* J. Bacteriol. **167**:138–147.

48. **Monod, M., S. Mohan, and D. Dubnau.** 1987. Cloning and analysis of *ermG,* a new macrolide-lincosamide-streptogramin B resistance element from *Bacillus sphaericus.* J. Bacteriol. **169**:340–350.

49. **Murphy, E.** 1985. Nucleotide sequence of *ermA,* a macrolide-lincosamide-streptogramin B determinant in *Staphylococcus aureus.* J. Bacteriol. **162**:633–640.

50. **Murphy, E., and S. Löfdahl.** 1984. Transposition of Tn*554* does not generate a target duplication. Nature (London) **307**:292–294.

51. **Novick, R. P., E. Murphy, T. J. Gryczan, E. Baron, and I. Edelman.** 1979. Penicillinase plasmids of *Staphylococcus aureus*: restriction-deletion maps. Plasmid **2**:109–129.

52. **Ounissi, H., and P. Courvalin.** 1981. Classification of macrolide-lincosamide-streptogramin B-type antibiotic resistance determinants. Ann. Inst. Pasteur (Paris) **132**:441–454.

53. **Phillips, S., and R. P. Novick.** 1979. A site specific repressor-controlled transposon in *Staphylococcus aureus.* Nature (London) **278**:476–478.

54. **Privitera, G., A. Dublanchet, and M. Sebald.** 1979. Transfer of multiple antibiotic resistances between subspecies of *Bacteroides fragilis.* J. Infect. Dis. **139**:97–101.

55. **Rasmussen, J. L., D. A. Odelson, and F. L. Macrina.** 1986. Complete nucleotide sequence and transcription of *ermF,* a macrolide-lincosamide-streptogramin B resistance determinant from *Bacteroides fragilis.* J. Bacteriol. **168**:523–533.

56. **Reig, M., M. G. Campello, and F. Baquero.** 1987. Inducible macrolide-lincosamide-streptogramin B resistance in *Bacteroides* species. Antimicrob. Agents Chemother. **31**:665–666.

57. **Rinckel, L. A., and D. C. Savage.** 1990. Characterization of plasmids and plasmid-borne macrolide resistance from *Lactobacillus sp.* strain 100-33. Plasmid **23**:119–125.

58. **Roberts, A. N., G. S. Hudson, and S. Brenner.** 1985. An erythromycin-resistance gene from an erythromycin-producing strain of *Arthrobacter sp.* Gene **35**:259–270.

59. **Rogers, E. J., N. P. Ambulos, Jr., and P. S. Lovett.** 1990. Complementarity of *Bacillus subtilis* 16S rRNA with sites of antibiotic-dependent ribosome stalling in *cat* and *erm* leaders. J. Bacteriol. **172**:6282–6290.

60. **Rollins, L. D., L. N. Lee, and D. J. LeBlanc.** 1985. Evidence for a disseminated erythromycin resistance determinant mediated by Tn*917*-like sequences among group D streptococci isolated from pigs, chickens, and humans. Antimicrob. Agents Chemother. **27**:439–444.

61. **Salaki, J. R., R. Balck, F. P. Tally, and J. W. Kislak.** 1976. *Bacteroides fragilis* resistant to the administration of clindamycin. Am. J. Med. **60**:426–428.

62. **Sandler, P., and B. Weisblum.** 1989. Erythromycin-induced ribosome stall in the *ermA* leader: a barricade to 5'-to-3' nucleolytic cleavage of the *ermA* transcript. J. Bacteriol. **171**:6680–6688.

63. **Schiller, J., N. Groman, and M. Coyle.** 1980. Plasmids in *Corynebacterium diphtheriae* and diphtheroids mediating erythromycin resistance. Antimicrob. Agents Chemother. **18**:814–821.

64. **Sebald, M., D. Bouanchaud, and G. Bieth.** 1975. Nature plasmidique de la résistance à plusieurs antibiotiques chez *C. perfringens* type A, souche 659. C.R. Acad. Sci. **280**:2401–2404.

65. **Shoemaker, N. B., E. P. Guthrie, A. A. Salyers, and J. F. Gardner.** 1985. Evidence that the clindamycin-erythromycin resistance gene of the *Bacteroides* plasmid pBF4 is on a transposable element. J. Bacteriol. **162**:626–632.

66. **Skinner, R., E. Cundliffe, and F. J. Schmidt.** 1983. Site of action of a ribosomal RNA methylase responsible for resistance to erythromycin and other antibiotics. J. Biol. Chem. **258**:12702–12706.

67. **Smith, C. J.** 1985. Characterization of *Bacteroides ovatus* plasmid pBI136 and structure of its clindamycin resistance region. J. Bacteriol. **161**:1069–1073.

68. **Smith, C. J., and F. L. Macrina.** 1984. Large transmissible clindamycin resistance plasmid in *Bacteroides ovatus.* J. Bacteriol. **2**:739–741.

69. **Stopler, T., and D. Branski.** 1986. Resistance of *Mycoplasma pneumoniae* to macrolides, lincomycin and streptogramin B. J. Antimicrob. Chemother. **18**:359–364.

70. **Tally, F. P., D. R. Snydman, M. J. Shimell, and M. H. Malamy.** 1982. Characterization of pBFTM10, a clindamycin-erythromycin resistance transfer factor from *Bacteroides fragilis.* J. Bacteriol. **151**:686–691.

71. **Tanaka, T., and B. Weisblum.** 1974. Mutant of *Staphylococcus aureus* with lincomycin- and carbomycin-inducible resistance to erythromycin. Antimicrob. Agents Chemother. **5**:538–540.

72. **Thakker-Varia, S., W. D. Jenssen, L. Moon-McDermott, M. P. Weinstein, and D. T. Dubin.** 1987. Molecular epidemiology of macrolide-lincosamide-streptogramin B resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. Antimicrob. Agents Chemother. **31**:735–743.

73. **Thakker-Varia, S., A. C. Ranzini, and D. T. Dubin.** 1985. Ribosomal RNA methylation in *Staphylococcus aureus* and *Escherichia coli*: effect of the "MLS" (erythromycin resistance) methylase. Plasmid **14**:152–161.

74. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1980. Properties of erythromycin-inducible transposon Tn*917* in *Streptococcus faecalis.* J. Bacteriol. **141**:1366–1374.

75. **Uchiyama, H., and B. Weisblum.** 1985. *N*-Methyltransferase of *Streptomyces erythreus* that confers resistance to the macrolide-lincosamide-streptogramin B antibiotics: aminoacid sequence and its homology to cognate R-factor enzymes from pathogenic bacilli and cocci. Gene **38**:103–110.

76. **Watanakunakorn, C.** 1976. Clindamycin therapy of *Staphylococcus aureus* endocarditis. Clinical relapse and development of resistance to clindamycin, lincomycin and erythromycin. Am. J. Med. **60**:419–425.

77. **Weisblum, B.** 1985. Inducible resistance to macrolides, lincosamides and streptogramin type B antibiotics: the resistance phenotype, its biological diversity, and structural elements that regulate expression—a review. J. Antimicrob. Chemother. **16**(Suppl. A):63–90.

78. **Weisblum, B., S. B. Holder, and S. M. Halling.** 1979. Deoxyribonucleic acid sequence common to staphylococcal and streptococcal plasmids which specify erythromycin resistance. J. Bacteriol. **138**:990–998.

79. **Wilkins, T. D., and T. Thiel.** 1973. Resistance of some species of *Clostridium* to clindamycin. Antimicrob. Agents Chemother. **3**:136–137.

80. **Yagi, Y., T. S. McLellan, W. A. Frez, and D. B. Clewell.** 1978. Characterization of a small plasmid determining resistance to erythromycin, lincomycin, and vernamycin B_α in a strain of *Streptococcus sanguis* isolated from dental plaque. Antimicrob. Agents Chemother. **13**:884–887.